

limitations. Claim 33 has been amended to delete the term "substantially" and to recite structural limitations as described in the specification i.e. "planar glass substrate," "oily liquid layer," and "aqueous droplet." Claim 34 has been amended to recite structural limitations as described in the specification i.e. "planar glass substrate," "oily liquid layer," and "aqueous droplet." Claim 34 has also been amended to insert "contact surface of" before planar "substrate." Further, Claim 34 has been amended to delete the term "substantially." Claim 36 has been amended to correct the dependency.

Claims 23, 24, 27, 28, 30, 31, and 35 have been canceled. Claims 21-22, 25-26, 29, 32-34, and 36 are pending.

CLAIM REJECTIONS UNDER 35 U.S.C. § 112, FIRST PARAGRAPH

Rejection of claims 21-39 under 35 U.S.C. §112, first paragraph

The Examiner rejected claims 21 - 39 as not enabled. The Examiner alleges the claims are too broad and require undue experimentation to make and use the claimed invention. Nevertheless, the Examiner states that the specification is enabling for a process for reducing evaporation during PCR comprising a glass substrate having an oily layer on top of a substrate and an aqueous droplet shot into the oily layer and onto the substrate where said oily layer surrounds all surfaces of said droplet which are not in contact with said substrate. In response, applicants have amended the claim language to claim the particular structures indicated in the specification, i.e. a planar substrate, oily liquid layer, and aqueous droplet, and providing the aqueous droplet to contact the planar substrate. Applicants submit that the claim amendments render the 35 U.S.C. §112, first paragraph rejections moot. Accordingly, applicants respectfully request withdrawal of this ground of rejection.

Rejection of claims 34-39 under 35 U.S.C. §112, first paragraph

Claims 34 - 39 have been rejected as not enabled. The Examiner asserts that the claims are written so broadly so as to encompass a myriad of reactions and that the specification teaches only the PCR reaction (page 3, last paragraph and pages 23 - 56). The Examiner further asserts that it would require undue experimentation to obtain the desired results in the claimed inventions. Applicants have amended the claims to include various structural limitations such as a "planar substrate," and "aqueous droplet," and an "oily layer." Applicants submit that specification and the claims are not limited to PCR reactions, as exemplified in the specification, but include other reactions where minute amounts of aqueous solutions are used, of which PCR is an example. Applicants respectfully submit that by providing the preferred oily liquid layers, one skilled in the art would not have to undertake undue experimentation to achieve the desired results (reduced evaporation) because the enumerated preferred oily liquid layers provide the desired results: protect the minute aqueous droplet from evaporation. In addition to providing the preferred oily layers, the present application provides an exemplary study in which one skilled in the art could use to test whether another particular oily layer would reduce evaporation and thus be suitable for use in the present invention. See page 20-21. Thus, applicants respectfully submit that the claims as amended satisfy the requirements of 35 U.S.C. § 112, first paragraph. Accordingly, applicants respectfully request withdrawal of this ground of rejection.

CLAIM REJECTIONS UNDER 35 U.S.C. § 112, SECOND PARAGRAPH

Claims 21 - 39 have been rejected as being indefinite. Claims 23, 24, 27, 28, 30, 31, and 35 have been canceled, thus rendering this ground of rejection, as it pertains to these claims, moot. With respect to the pending claims, the applicants have amended them according to the Examiner's suggestions as set forth in points 7a-7j.. Accordingly, Applicants respectfully request withdrawal of this ground of rejection.

Claim rejections under 35 U.S.C. § 103(a) over Gelfand et al.

Rejection of 21-36 and 39

Claims 21 -36, and 39 have been rejected under 35 U.S.C. 103(a) as obvious over Gelfand et al. (U.S. Patent No. 5,410,652). The Examiner alleges that Gelfand et al. disclose a process of reducing evaporation comprising a substrate, liquid layer and a minute droplet in contact with said substrate. According to the Examiner, Gelfand's process includes layering a mineral oil over the surface of said minute droplet that is not in contact with said substrate (Col. 33, lines 1-20). The Examiner also alleges that it would have been known to one of ordinary skill in the art that it is advantageous to apply the liquid layer before adding the minute droplet in order to reduce the risk of cross-contamination between samples and eliminate applying the liquid layer sample-by-sample. Applicants submit that it would not have been obvious to one skilled in the art to extend Gelfand to the present invention. The present invention is directed to a process for reducing evaporation or a process for conducting a reaction in a minute droplet protected from evaporation in which a planar substrate is provided. There is no teaching nor suggestion in Gelfand et al. of providing a planar substrate. Gelfand et al. teach the use of a centrifuge tube, which is conical in shape. Further, the process of the present invention is directed to providing an oily layer onto a planar substrate and then providing a minute aqueous droplet into the oily layer to contact the planar substrate to reduce evaporation. Applicants submit that there is no teaching nor suggestion of such in Gelfand et al. Accordingly, applicants respectfully request withdrawal of this ground of rejection as Gelfand et al. do not render the claims obvious.

Regarding Claim 21, Gelfand et al. teach the addition of an aqueous solution to a microfuge tube band before applying an oil overlay and spinning the mix in a microfuge "to separate the oil layer from the reaction mix." (Col 33, lines 19-20). Gelfand et al. provide a method for minimizing contamination by using one tube for a one enzyme procedure to

eliminate the need to open the tube to add reagents before the amplification. (Col 5, lines 32 - 36). Applicants respectfully submit that Gelfand et al. do not teach nor suggest adding an oily liquid layer to a planar substrate to reduce evaporation. Gelfand et al. do not teach providing a minute aqueous droplet to contact a planar substrate to immobilize the droplet. Thus, applicants submit that Gelfand et al. do not render claim 21 obvious. Accordingly, applicants request withdrawal of this ground of rejection.

With respect to claim 22, the Examiner alleges that it would be obvious to one of ordinary skill in the art to use microfuge tubes that are water repellent. Applicants submit that Gelfand et al. teach the use of a microfuge tube and do not teach nor suggest the use of a planar substrate with water repellency. In contrast to Gelfand et al's microfuge tube, the present invention provides a planar substrate that is water repellent in order to minimize spreading of the minute droplet on a planar substrate. The water repellent planar substrate allows the aqueous minute droplet to have a smaller area of contact with the planar substrate, and thus allows the minute droplet to rise in the thickness direction of the liquid layer, which in turn, provides a longer optical path and allows for easier optical detection. See page 5 of the specification. Thus, it would not be obvious to one of ordinary skill in the art to modify the method by replacing the microfuge tube with a planar substrate that is water repellent because water repellency is not relevant to prevent spreading in a microfuge tube. Accordingly, applicants request withdrawal of this ground of rejection.

With regard to claims 23 and 24, the cancellation of these claims renders this ground of rejection moot. Accordingly, applicants request withdrawal of this ground of rejection.

Regarding claim 25, the Examiner states that Gelfand et al. teach layering a liquid layer of mineral oil over an aqueous solution, wherein said liquid layer consists of mineral oil. In contrast, the present invention is directed to providing a planar substrate and an oily liquid layer, and providing a minute aqueous droplet, which is substantially

immiscible with said oily liquid layer, into the oily liquid layer whereby the aqueous minute droplet contacts the planar substrate. Applicants respectfully submit that Gelfand et al. do not teach nor suggest adding an oily liquid layer to a planar substrate and providing a minute aqueous droplet that is substantially immiscible with said oily liquid layer, whereby the aqueous minute droplet contacts the planar substrate. Accordingly, applicants request withdrawal of this ground of rejection.

Regarding Claim 26, the Examiner states that although Gelfand et al. do not teach a injection method for said aqueous droplet into said liquid layer, it would be obvious to one of ordinary skill in the art to modify the method of Gelfand et al. to include an injection delivery of the said aqueous droplet. As discussed above, Gelfand et al. do not teach nor suggest adding an oily layer to a planar substrate and providing a minute aqueous droplet to contact a substrate to immobilize said droplet. Rather, Gelfand et al. teach layering an oily liquid over an aqueous solution contained in a microfuge tube. Thus, applicants submit that Gelfand et al. do not teach or suggest delivering an aqueous droplet to contact a planar substrate.

Accordingly, applicants request withdrawal of this ground of rejection.

Regarding claims 27 and 28, the cancellation of these claims renders this ground of rejection moot. Accordingly, applicants request withdrawal of this ground of rejection.

Regarding Claim 29-31, the Examiner asserts that Gelfand et al. teach covering the liquid layer with a microfuge cover. Applicants respectfully submit that Gelfand et al. do not teach nor suggest providing a covering over an oily liquid layer that surrounds all surfaces of a minute aqueous droplet that are not in contact with the planar substrate or the covering. Gelfand et al. merely teach providing a microfuge cap to cover said liquid layer contained in a microfuge tube. The present invention teaches that by providing a cover to contact the upper surface of a minute droplet, one can elongate the optical path for facilitating optical measurements. Furthermore, Gelfand et al. does not teach nor suggest applying a cover in

contact with said liquid layer contained on a planar substrate to elongate an optical path. Accordingly, applicants request withdrawal of this ground of rejection.

Regarding claims 33 and 34, Gelfand et al. teach the addition of an aqueous solution to a microfuge tube before applying an oil overlay and microfuge tube cap as a method for minimizing evaporation. Gelfand et al. do not teach nor suggest adding an oily liquid layer to a planar substrate to reduce evaporation nor does it teach providing a minute droplet to contact a substrate to immobilize the droplet. Furthermore, Gelfand et al. do not teach nor suggest applying a cover in contact with said liquid layer contained on a planar substrate to elongate an optical path. As such, applicants submit that Gelfand et al. do not render the present claims obvious. Accordingly, applicants request withdrawal of this ground of rejection.

Regarding Claim 36, Gelfand et al. teach adding an aqueous solution comprising DNA to a microfuge tube before applying an oily liquid layer. As discussed above, Gelfand et al. do not teach nor suggest providing an oily liquid layer to a planar substrate nor does it teach providing a minute aqueous droplet comprising DNA to contact a planar substrate. As such, applicants submit that Gelfand et al. do not render the present claims obvious.

Accordingly, applicants request withdrawal of this ground of rejection.

Regarding Claim 39, the Examiner alleges it would be obvious to modify Gelfand et al.'s teaching of the use of 75 μ l oil overlay to prevent evaporation using a microfuge tube substrate to use a liquid layer with a thickness of 100 μ m or less. Applicants submit that it would not have been obvious to modify Gelfand et al.'s teaching of an oil overlay of 75 μ l in a microfuge tube to arrive at the present invention directed to a planar substrate having an oily liquid layer of a thickness of 100 μ m or less where an aqueous minute droplet is provided into the oily layer to contact a surface of the planar substrate. Accordingly, applicants request withdrawal of this ground of rejection.

**CLAIM REJECTIONS UNDER 35 U.S.C. § 103(A) OVER GELFAND ET AL. IN
VIEW OF SAMBROOK ET AL.**

Rejection of Claims 37 and 38

The Examiner asserts that although Gelfand et al. do not teach the method wherein the contact surface between said substrate and said droplet comprises the enzyme absorption preventing agent and the contact surface comprises a bovine serum albumin coating, it would be obvious to one skilled in the art to utilize the methods of using Denhardt's reagents as taught by Sambrook to modify the method of Gelfand et al. As previously discussed, Gelfand et al. do not teach nor suggest an oily liquid layer surrounding all surfaces of a minute aqueous droplet not in contact with the planar substrate. Additionally, applicants further submit that Sambrook et al. fail to teach providing a liquid layer surrounding all surfaces of a minute aqueous droplet not in contact with the planar substrate. Sambrook et al. merely teach the use of various reagents, such as Denhardt's reagent, to block non-specific reactions. Applicants, therefore, submit that the combination of Gelfand et al. in view of Sambrook et al.'s disclosures fails to teach or suggest the present invention.

Applicants respectfully submit that Gelfand et al. in view of Sambrook et al. do not render the claims obvious. Accordingly, applicants request withdrawal of this ground of rejection.

CONCLUSION

In view of the foregoing amendments and remarks, it is firmly believed that the subject invention is in condition for allowance, which action is earnestly solicited.

The Office is hereby authorized to charge Deposit Account No. 11-0600 with any additional fees required by this paper or credit any overpayment.

If the Examiner believes, for any reason, that personal communication will expedite

prosecution of this application, the Examiner is invited to telephone the undersigned directly at (202) 220-4258.

Prompt and favorable consideration of this Amendment is respectfully requested.

KENYON & KENYON

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